

New England Biolabs Certificate of Analysis

Product Name: *Immobilized T4 DNA Ligase*
Catalog Number: *M0569S*
Concentration: *10 mg/ml*
Packaging Lot Number: *10241822*
Expiration Date: *05/2026*
Storage Temperature: *-20°C*
Storage Conditions: *10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, (pH 7.4 @ 25°C)*
Specification Version: *PS-M0569S/L v1.0*


Immobilized T4 DNA Ligase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0569SVIAL	Immobilized T4 DNA Ligase	10241821	Pass
B0202SVIAL	T4 DNA Ligase Reaction Buffer	10230730	Pass

Assay Name/Specification	Lot # 10241822
Endonuclease Activity (Nicking) A 50 µl reaction in NEBuffer 1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 10 µg of Immobilized T4 DNA Ligase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
Functional Testing (Bead Concentration) In triplicate reactions, Immobilized T4 DNA Ligase (500 µl) was delivered to tared microcentrifuge tubes. The beads are pelleted using a magnetic separation rack and washed sequentially 3 times with 1 mL of Milli Q water using the magnetic separation rack to pellet the beads and remove the supernatant each time. The microcentrifuge tubes containing the pelleted, desalted beads were incubated at 95°C for 4 hours and weighed to determine the concentration of the beads to be 10.0 ± 0.3 mg/ml.	Pass
Functional Testing (Magnetic Beads, Leaching) Immobilized T4 DNA Ligase (100 µl) was incubated in storage buffer for 24 hours at room temperature. The beads were pelleted using a magnetic separation rack and the supernatant boiled in SDS-PAGE buffer for 5 minutes at 100°C. No Immobilized T4 DNA Ligase was detected in the supernatant as determined by an absence of bands visualized using Tris-Glycine gel electrophoresis with Coomassie Blue detection.	Pass
Functional Testing (Targeted Ligation)	Pass

Assay Name/Specification	Lot # 10241822
<p>A 20 µl reaction in 1X Quick Ligation Buffer containing 20 pmol of a FAM-labeled RNA and an excess of duplex DNA adaptor with 1 µg of Immobilized T4 DNA Ligase incubated for 10 minutes at 25°C results in ≥90% ligation as determined by capillary electrophoresis.</p>	
<p>Non-Specific DNase Activity (16 Hour) A 50 µl reaction in NEBuffer 1 containing 1 µg of CIP-treated Lambda-HindIII DNA and a minimum of 10 µg of Immobilized T4 DNA Ligase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>	Pass
<p>RNase Activity (Extended Digestion) A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of Immobilized T4 DNA Ligase is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	Pass
<p>qPCR DNA Contamination (E. coli Genomic) A minimum of 10 µg of Immobilized T4 DNA Ligase is screened for the presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome.</p>	Pass

This product has been tested and shown to be in compliance with all specifications.

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11 Jun 2024



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13 Jun 2024